Two Ways to Get Mad at Kinetochores

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The spindle assembly checkpoint ensures that mitotic cells only segregate their sister chromatids once all chromosomes are attached via kinetochores by microtubules of the mitotic spindle. Reporting in *Developmental Cell*, Silió et al. (2015) show that in human cells the signaling cascade controlling the checkpoint operates through two separate branches.

The spindle assembly checkpoint (SAC) ensures that chromosomes are correctly attached via kinetochores by the microtubules of the mitotic spindle before sister-chromatid segregation. The checkpoint responds to the presence of unattached or incorrectly attached chromosomes by recruiting the Mad1:Mad2 complex onto kinetochores. This recruitment leads to the formation of a diffusible signal that inhibits the anaphase-promoting complex and its co-factor Cdc20, delaying anaphase onset by preventing sister-chromatid segregation and Cyclin B degradation (Foley and Kapoor, 2013). One fundamental question in the field is how Mad1:Mad2 are recruited to improperly attached kinetochores; the molecular mechanism by which this occurs remains unclear. The initiating step in this signaling cascade is recruitment of the kinase Mps1, whose binding to kinetochores is inhibited by the presence of microtubule plus ends (Hiruma et al., 2015). Studies in yeast have suggested a linear pathway, in which Mps1 phosphorylates the kinetochore protein Knl1 to allow the recruitment of the Bub3:Bub1 complex, which in turn is phosphorylated by Mps1 to act as a loading platform for Mad1:Mad2 (London and Biggins, 2014). In this issue of *Developmental Cell*, Silió et al. (2015) now show that in human cells the Knl1/Bub3/Bub1 kinetochore recruitment pathway for Mad1:Mad2 is not unique, but is complemented by a second pathway that depends on the Rod/ZW10/Zwilch (RZZ) kinetochore complex.

The results of this study indicate that Knl1 depletion is sufficient neither to abolish SAC signaling in response to unattached kinetochores, nor to prevent the loading of Mad1:Mad2 onto those same unattached kinetochores, even though it abrogates the loading of Bub1. This indicates the existence of a second recruiting pathway for Mad1:Mad2, consistent with the recent finding that human Bub1 is not absolutely required for Mad1 binding to kinetochores, even though it accelerates its recruitment onto this structure (Vleugel et al., 2015). Silió and colleagues (2015) demonstrate that this second pathway depends on RZZ, a kinetochore complex originally identified as a SAC component in *Drosophila melanogaster* that is not found in fungi (Basto et al., 2000). Depletion of both Knl1 and RZZ abrogates the recruitment of Mad1:Mad2 to kinetochores and impairs the SAC response, indicating that both pathways act in a complementary manner.

These exciting findings raise one caveat and a number of key future questions. First, there is the caveat that Silió et al. (2015) relied on small interfering RNA (siRNA)-mediated depletions to investigate the role of Knl1 in SAC signaling. While Knl1 depletion was very efficient (>95%), one has to bear in mind that structural kinetochore proteins required for the SAC, such as the Mps1-recruiting subunits Ndc80 and Nuf2R, need to be depleted more than 100-fold to disrupt this checkpoint (Hiruma et al., 2015; Meraldi et al., 2004). At this stage, it is therefore not possible to completely exclude that Knl1 is essential for SAC signaling in response to unattached kinetochores. To remove this last uncertainty, it will be essential to knock out Knl1, e.g., by using the CRISPR/CAS technology; however, this might prove difficult because a Knl1 knockout might be lethal for cells, as Knl1 and its interaction partners are also involved in the establishment of kinetochore-microtubule attachments.

A second important question is why metazoan cells would have two separate branches for SAC activation. Because Knl1-depleted cells activate the SAC in the presence of unattached kinetochores but fail to respond to unaligned kinetochores, Silió et al. (2015) propose that the RZZ complex is required for the response to unattached kinetochores, while the Knl1/Bub1/Bub3 branch might be important for unaligned kinetochore pairs. This would allow the SAC to respond to more than one type of defect, ensuring a higher rate of chromosome segregation fidelity. Therefore, one key step will be to determine the precise microtubule-attachment configuration of the unaligned kinetochores in Knl1-depleted cells, possibly by electron microscopy. In parallel, a careful analysis of the defects arising after loss of RZZ alone will be necessary in order to better understand which type of defects this complex responds to. RZZ is present in animal and plant cells (Starr et al., 1997), indicating that it was selectively lost in fungi. It will therefore be interesting to understand how Knl1, Bub3, and Bub1 have, in fungi, taken over the role played by RZZ in metazoans. Because spindle poles are located in close proximity to kinetochores in fungi, kinetochores spend little time unattached when cells enter mitosis. For this reason, the RZZ complex may not be needed to delay anaphase onset in yeast. A third crucial question is how and to what extent the Knl1/Bub3/Bub1 and RZZ branches of the SAC cross-talk. While the human RZZ complex is essential for SAC signaling in the absence of Knl1, Bub3, or Bub1 at kinetochores, human Bub1 itself is essential for the SAC (Meraldi and Sorger, 2005). This suggests that a non-kinetochore pool of Bub1 or a small non-detectable pool of Bub1 contributes to the RZZ pathway, consistent with a recent study showing that Bub1 contributes to the recruitment of RZZ to kinetochores (Zhang et al., 2015).

Finally, one of the most striking findings of this present study is the fact that, despite very similar depletion efficiencies, Knl1 depletion leads to much more severe chromosome-alignment defects in the cancerous cervix HeLa cells than in the non-transformed retina-derived RPE1 cell line. The authors suggest as possible explanations the difference in ploidy between the cells (76–80 chromosomes versus 46)—which might facilitate chromosome alignment in RPE1 cells, as fewer chromosome need to be attached by the spindle—or a fundamental difference in kinetochore function. This latter hypothesis is supported by the fact that depletion of Knl1 leads to a strong reduction in RZZ levels at kinetochores in HeLa cells while barely affecting this complex in RPE1 cells. If kinetochore functions were to fundamentally differ between cancerous and non-cancerous cells, Knl1 and its interaction partners might offer an ideal target for anti-cancer treatments, as any compound blocking Knl1 function might preferentially impair chromosome segregation in cancer cells. The validation of such an approach will, however, require a much more systematic analysis of multiple cancerous and non-cancerous cell lines, which ideally should be derived from the same tissue.

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